CLAIMS

What is claimed is:

1. A method of labeling peptides, comprising the steps of:

a) obtaining peptides formed from proteins digested by proteolyic enzymes selected from trypsin, chymotrypsin, Lys-C endoprotease, Glu-C endoprotease, an endoprotease that cleaves at the C-terminal side of an amino acid residue on a protein, or a mixture of two or more of these proteolytic enzymes; and thereafter

b) incorporating isotopic atoms into said peptides in the catalytic presence of proteolyic enzymes selected from trypsin, chymotrypsin, Lys-C endoprotease, Glu-C endoprotease, an endoprotease that cleaves at the C-terminal side of an amino acid residue on a protein, or a mixture of two or more of these proteolytic enzymes.

2. The method of claim 1 wherein the proteolytic enzymes or mixture of enzymes in step a) is the same as the proteolytic enzymes or mixture of proteolytic enzymes in step b).

3. The method of claim 2 wherein the proteolytic enzymes in step a) and in step b) are trypsin.

4. The method of claim 2 wherein the proteolytic enzymes in step a) and in step b) are a mixture of Glu-C and trypsin.

5. The method of claim 1 wherein the proteolytic enzymes or mixture of enzymes in step a) are different from the proteolytic enzymes or mixture of proteolytic enzymes in step b).

6. The method of claim 5 wherein the proteolytic enzymes in step a) are Lys-C and in step b) are trypsin.

7. The method of claim 1 wherein said the proteolytic enzymes in step a) and in step b) can be obtained from any species or from thermophilic microorganisms.

8. The method of claim 1 wherein one or two isotopic atoms are incorporated into a peptide and where said isotopic atoms are ^{18}O atoms.

9. The method of claim 8 wherein step of incorporating isotopic atoms into said peptides is conducted in $H_2{}^{18}O$.

10. The method of claim 1, further comprising mixing labeled peptides obtained from the step of incorporating of isotopic atoms with peptides that have not been labeled to obtain a mix of labeled and unlabeled peptides.

11. The method of claim 10, further comprising analyzing the mix of labeled and unlabeled peptides by mass spectrometry.

12. The method of claim 11, further comprising calculating a ratio of labeled peptides to unlabeled peptides with formula (I) or (II)

$$\text{ratio} = \{I_4 - (M_4/M_0)) \times I_0 - (M_2/M_0) \times [I_2 - (M_2/M_0) \times I_0] + [I_2 - (M_2/M_0) \times I_0]\}/I_0 \quad \text{(I)}$$

$$\text{ratio} = \{I_4 - (M_4/M_0)) \times I_0 - (M_2/M_0) \times [I_2 - (M_2/M_0) \times I_0] + 1/2[I_2 - (M_2/M_0) \times I_0]\}/I_0 \quad \text{(II)};$$

where I_0, I_2 and I_4 are observed peak areas for a monoisotopic peak for peptides without ^{18}O label, a peak 2Da higher and peak 4Da higher, and M_0, M_2 and M_4 are the theoretical peak areas for the monoisotopic peak for a peptide with a known composition, a a peak 2Da higher and peak 4Da higher, respectively.

13. The method of claim 11, wherein said mass spectrometry is nanospray, electrospray, LC-MS, LC-MS-MS or matrix-assisted IR or UV laser desorption ionization, high vacuum, atmospheric or low pressure, on a quadropole, quadropole ion trap, time-of-flight, ion cyclotron resonance, magnetic sector ion analyzer or a combination thereof.

14. The method of claim 1, wherein said step of incorporating isotopic atoms results in peptides in the catalytic presence of proteolytic enzymes being labeled greater than 90% with one or two ^{18}O atoms.

15. The method of claim 1, further comprising a step of storing the peptides overnight after step a) and before step b).

16. A method of labeling peptides, comprising the steps of:

digesting proteins with proteolytic enzymes in an $H_2{}^{16}O$ environment to obtain peptide fragments of said proteins, where said proteolyic enzymes are selected from trypsin, chymotrypsin, Lys-C endoprotease, Glu-C endoprotease, an endoprotease that cleaves at the C-terminal side of an amino acid residue on a protein, or a mixture of two or more of these proteolytic enzymes; and then

labeling at least a first portion of said peptide fragments by incorporating ^{18}O atoms into said at least first portion of said peptide fragments in an $H_2{}^{18}O$ environment and in the catalytic presence of proteolyic enzymes selected from trypsin, chymotrypsin, Lys-C endoprotease, Glu-C endoprotease, an endoprotease that cleaves at the C-terminal side of an amino acid residue on a protein, or a mixture of two or more of these proteolytic enzymes.

17. The method of claim 16, further comprising a step of storing the peptides overnight after the step of digesting proteins and before the step of labeling.

18. The method of labeling peptides of claim 17, further comprising the step of:

mixing a second unlabeled portion of peptide fragments with the labeled first portion of peptide fragments.

19. A method of quantitatively analyzing proteins, comprising the steps of:

obtaining a protein sample;

then dissolving the protein sample in a solution of buffer or chaotropic agent;

then forming a peptide sample by digesting the protein sample by adding one or more of trypsin, chymotrypsin, Lys-C endoprotease, Glu-C endoprotease, and/or an endoprotease that cleaves at the C-terminal side of an amino acid residue on a protein;

then incubating the peptide sample at least overnight;

then retrieving the peptide sample;

then contacting at least a first portion of the peptide sample with said one or more of trypsin, chymotrypsin, Lys-C endoprotease, Glu-C endoprotease, and/or an endoprotease that cleaves at the C-terminal side of an amino acid residue on a protein and with $H_2{}^{18}O$ to label the at least first portion of the peptide sample with ^{18}O.

20. The method of labeling peptides of claim 19, further comprising the steps of:

preparing a second unlabeled portion of said peptide samples by contacting said second portion of the peptide sample with said one or more of trypsin, chymotrypsin, Lys-C endoprotease, Glu-C endoprotease, and/or an endoprotease that cleaves at the C-terminal side of an amino acid residue on a protein and with $H_2{}^{16}O$; and then

mixing a second unlabeled portion of said peptide samples with the labeled first portion of the peptide sample;

then calculating a ratio of labeled peptides to unlabeled peptides with formula (I) or (II)

$$\text{ratio}= \{I_4- (M_0/M))\times I_0-(M_2/M_0) \times [I_2-(M_2/M_0) \times I_0] + [I_2 -(M_2/M_0) \times I_0]\}/I_0 \qquad (I)$$

$$\text{ratio}= \{I_4- (M_0/M))\times I_0-(M_2/M_0) \times [I_2-(M_2/M_0) \times I_0] + 1/2[I_2 -(M_2/M_0) \times I_0]\}/I_0 \qquad (II)$$

where I_0, I_2 and I_4 are observed peak areas for a monoisotopic peak for peptides

without ^{18}O label, a peak 2Da higher and peak 4Da higher, respectively, and M_0, M_2 and M_4 are the theoretical peak areas for the monoisotopic peak for a peptide with a known composition, a peak 2Da higher and peak 4Da higher, respectively.